Conclusions: Global gene expression analysis of dedifferentiated chondrocytes relative to native cartilage and other mesenchyme-derived tissue would suggest that loss of functional phenotype in monolayer culture encourages the up-regulation of genes associated with pre-hypertrophic chondrocytes during development.

393  
A FUNCTIONAL INVESTIGATION OF A RARE VARIANT IN THE PROMOTER OF THE OA-ASSOCIATED LOCUS GDF5 AND THE DISCOVERY OF A TRANS-FACTORY INTERACTING WITH THE VARIANT SITE

A.W. Dodd, J. Loughlin. Newcastle Univ., Newcastle upon Tyne, United Kingdom

Purpose: The rs143383 SNP within the 5’UTR of the growth/differentiation factor 5 gene GDF5 is one of the most robust association signals for large-joint OA. The SNP acts by altering the level of GDF5 expression and this effect is modulated by a second 5’UTR SNP, rs143384. We recently completed a deep sequencing of the gene to identify novel rare variants that may also be functional, with one variant found within the promoter of GDF5, -41bp from the transcription start site. In this report we set out to determine what functional effect this variant is having on GDF5 expression, including the identification of the trans-acting factors binding to the variant site.

Methods: A 403bp fragment encompassing the proximal promoter and part of the 5’UTR of GDF5 was cloned into the pcG3 Basic Luciferase reporter plasmid. Following molecular haplotyping, we created haplotype combinations between the -41bp variant alleles and the rs143383 and rs143384 alleles. We transfected these plasmids into SW1353 chondrocytes and into MG63 osteosarcoma cells and measured luciferase activity. Electrophoretic mobility shift assays (EMSA) were then performed to identify the trans-acting factors binding to the -41bp variant, and to compare their binding affinity to the two variant alleles. A super-shift assay, using a trans-factor specific antibody, was then carried out to confirm specific protein binding.

Results: The transfection experiment revealed that the mutant A-allele of the -41bp variant mediated a significant increase in gene expression compared to the wildtype C-allele, independent of genotype at rs143383 or rs143384. The greatest difference between the -41bp alleles was observed in the MG63 cell line, with a 36% increased expression of the A-allele relative to the C-allele (p=0.0009). Our EMSA and supershift analyses revealed that the YY1 transcription factor binds to the variant site and that this binding is more avid for the mutant A-allele, implying that YY1 is an activator of GDF5 expression.

Conclusions: A variant discovered by deep sequencing of GDF5 has been shown to be functional and to influence gene expression independent of the common functional polymorphisms rs143383 and rs143384. The variant increases expression by increasing the binding of the transcription factor YY1. Our study has therefore identified a novel functional region of this important OA susceptibility locus and revealed a trans-acting factor that regulates GDF5 expression.

394  
CPS SITES OF OSTEARTHRITIS SUSCEPTIBILITY GENE DIO2 ARE DIFERENTIALLY METHYLATED IN ARTHRITIC COMPARED TO PRESERVED CARTILAGE.

W. den Hollander, S.D. Bos, Y.F. Ramos, N. Lakenberg, R. van der Breggen, B.J. Duijnsveld, R.G. Nellisen, E.P. Slagboom, I. Meulenbelt. LUMC, Leiden, Netherlands

Purpose: Through genome wide approaches genetic variation at deiodinase type II (D2) gene (DIO2) was identified as compelling osteoarthritis (OA) susceptibility gene. Furthermore, studies showed that DIO2 gene expression is up regulated in osteoarthritic as compared to healthy cartilage, which might be explained by an allelic imbalance of the DIO2 mRNA in cartilage in which we observed a ~ 30% higher presence of the OA-associated C allele relative to the T allele of the intragenic DIO2 SNP rs225014 in heterozygous carriers. In the current study, we investigate whether the expression of DIO2 is modulated epigenetically by DNA methylation at CpG dinucleotides in the promoter, in transcription factor binding sites (TFBS) and/or in an intragenic putative CTCF binding site.

Methods: We collected both preserved and osteoarthritic cartilage (determined macroscopically) from OA patients undergoing total joint arthroplasty (N=26, age range 44-80 years) of the hip or knee. Cartilage was snap frozen in liquid nitrogen and stored at ~80 °C. Using a Retsch MM200 sample disintegrator cartilage was powdered prior to DNA and RNA isolation. DNA was isolated using Promega Wizard Genomic DNA Purification kit and RNA was isolated by trizol addition and Qiagen RNeasy columns. Bisulfite treatment of isolated DNA was performed with Zymo EZ DNA Methylation kit. Eight amplicons covering 16 CpG dinucleotides were designed using MethPrimer; amplicons were designed to encompass the promoter, TFBSs and a putative CTCF binding site. Amplicons were PCR amplified and quantitatively assessed for CpG methylation using a mass spectrometry based method (Epityper, Sequenom). Statistical analysis was performed using a two tailed, paired t-test.

Results: We examined the differences in methylation state of CpG dinucleotides of DIO2 in matched OA affected and preserved cartilage samples. Of five amplicons analyzed thus far, at least three CpG dinucleotides located in a TFBS upstream of the promoter, were differentially methylated in preserved compared to arthritic cartilage (P=0.002, P=0.031 and P=0.023, paired t-tests). Of these, two CpG dinucleotides show a significant reduction in methylation, whereas one CpG site was hypermethylated in osteoarthritic cartilage as compared to preserved cartilage.

Conclusions: Methylation states of CpG nucleotides located in TFBS upstream of the DIO2 promoter differ significantly between preserved cartilage and osteoarthritic cartilage ex vivo. Follow up of the methylation status of these CpG dinucleotides, in relation to DIO2 expression, will further elucidate the putative regulatory properties. These preliminary analyses indicate that there is a disease related differential methylation state of three CpG dinucleotides located in essential DIO2 regulatory DNA motifs.

395  
THE MTDNA HAPLOGROUPS RELATED WITH THE MITOCHONDRIAL UNCOUPLING MECHANISM ARE PROTECTIVE FACTORS AGAINST OA IN SPAIN AND UK


Purpose: Oxidative stress and ROS production are key factors in the development of OA. Some reports have shown that those mtDNA haplogroups related with the mild uncoupling of the mitochondrial oxidative phosphorylation system (OXPHOS), such as haplogroups J and T, are less prone to produce reactive oxygen species (ROS). Taking into account that the mtDNA haplogroup J is a protective factor against OA in Spanish populations, the aim of this work is to compare the frequency distribution of the mtDNA haplogroups in OA patients and healthy controls between United Kingdom (UK) and Spain.

Methods: We used the single base extension (SBE) assay combined with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to obtain the European mtDNA haplogroups in 1471 OA patients and 406 healthy controls from Spain, and 453 OA patients and 280 healthy controls from UK (samples kindly provide by Prof. J. Loughlin). Some of the differential single nucleotide polymorphisms (SNPs) haplogroup J-related between both populations were also analyzed using the SBE assay. The resulting frequencies were analyzed with SPSS software (v.18) following appropriate statistical analyses.

Results: The mtDNA haplogroup J appeared significantly underrepresented in OA patients from the north of Spain when compared with healthy controls (OR=0.614; 95% CI: 0.426-0.884; p=0.008). Individuals from UK carrying the mtDNA haplogroup J showed a significantly decreased risk of OA (OR=0.581; 95% CI: 0.365-0.926; p=0.021). The mtDNA haplogroup J bordered the statistical significance towards a decreased presence in healthy controls from UK (OR=1.686; 95% CI: 0.929-3.061; p=0.083). The analysis of the differentially expressed haplogroup J-related SNPs in Spain and UK, m14798t>c, m15257g>a and m3394t>c showed that the SNP m3394t>c (associated with uncoupling of OXPHOS and with less production of ROS) appeared significantly underrepresented in the UK cohort (p=0.029).

Conclusions: The mitochondrial uncoupling mechanism, derived from the mtDNA haplogroups J and T, could be a protective factor against OA in